Meconium Mineral Content in Small for Gestational Age Neonates

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ABSTRACT

The mineral concentration of meconia of small for gestational age (SGA) newborns were compared with those of appropriate for gestational age (AGA) newborns of similar gestational ages (GA) to determine whether differences may provide clues of possible nutritional deficits of SGA infants, given that levels of meconium minerals could indicate the use of minerals by the fetus and the sufficiency of the maternal supply of minerals. Twenty-one SGA and 24 AGA newborns were included. Eleven SGA and 15 AGA were ≤ 35 weeks GA. Ten SGA and nine AGA infants were ≥ 36 weeks GA. All meconia from each neonate was processed and assayed for iron, zinc, copper, manganese, calcium, magnesium, and phosphorus. In the ≤ 35-week subgroups, the SGA infants had lower meconium iron and manganese concentrations than that of the AGA. Among ≥ 36-week newborns, SGA infants had a higher birthweight-adjusted copper concentration than AGA infants, but no differences were observed for the remaining elements. Lower iron and manganese meconium in ≤ 35-week SGA infants may reflect either a greater use or a decreased maternal supply. The higher birthweight-adjusted meconium copper in the ≥ 36-week SGA infants may be due to a comparatively reduced fetal use or increased maternal supply. These data may assist in clarifying potential mechanisms affecting intrauterine growth and/or potential nutrient deficits in the neonatal period.

KEYWORDS: Meconium minerals, prematurity, growth restriction

The fetus is totally dependent upon the mother for its supply of minerals essential for growth and development. The placental transport of minerals is highly complex and is determined by maternal, fetal, and placental factors that are specific for each element.[1–3] Assessment of mineral accretion by the fetus during gestation might require invasive procedures or a postmortem analysis of aborted fetuses. In an effort to evaluate the mineral stores of the fetus, the composition of meconium offers interesting possibilities. Meconium is the first excretion from the alimentary canal of the newborn. It is formed by gastrointestinal secretions, mucus, bile, cellular debris, lanugo hair, vernix caseosa, and occasionally occult blood. Because meconium starts collecting in the alimentary canal of the fetus between the 14th to 16th weeks of gestation through birth, it may reflect mineral transport to the fetus during an extended period of fetal growth when the quantitative demands for tissue formation greatly increase,[4] and is the phase of most mineral accretion by the fetus.[5] The mineral content of meconium may thus represent the fraction of minerals received from the mother, not assimilated by

American Journal of Perinatology, Volume 22, Number 5, 2005. Address for correspondence and reprint requests: Raul A. Wapnir, Ph.D., M.P.H., North Shore–Long Island Jewish Research Institute, North Shore–Long Island Jewish Health System, 350 Community Drive, Manhasset, NY 11030. [1]Division of Neonatal-Perinatal Medicine, Schneider Children's Hospital at North Shore, Manhasset, New York; [2]North Shore–Long Island Jewish Research Institute, North Shore–Long Island Jewish Health System, Manhasset, New York. Tel: +1(212) 584-4662. DOI 10.1055/s-2005-870660. Published online June 15, 2005. 0735-1631,p;2005,22,05,259,263,ftx,en;ajp41350x.

the fetus, and finally excreted soon after birth. The small concentration of calcium present in the amniotic fluid could also contribute to a very limited extent. Individual element concentration in meconium may provide an index of specific mineral use by the fetus, as well as a record of the adequacy of the minerals received from the mother as regulated by the placenta.[6–8]

In a previous study conducted in our laboratory, we have shown that the birthweight-adjusted concentrations of iron, copper, calcium, and phosphorus in meconium were higher in premature than in term neonates, whereas zinc, manganese, and magnesium did not change with gestational age (GA).[9] In a subsequent study comparing meconium minerals between dizygotic twins, the higher mineral content of the heavier twin supported the view that the weight-advantaged fetus in a weight-discordant twin pair had benefited from a greater access to maternally supplied mineral nutrients.[10]

Small-for-gestational-age (SGA) neonates have experienced intrauterine growth restriction. There are several factors influencing fetal growth. Most prominent maternal factors include her nutritional status and medications, as well as conditions such as cyanotic heart disease, vascular and renal insufficiency, and diabetes mellitus. Placental factors encompass reduced placental size and surface area, structural abnormalities, and uteroplacental blood flow. Fetal factors include inborn errors of metabolism, toxins, and chromosomal abnormalities.[11,12] All of the above-mentioned factors may be responsible for either limited availability of nutrient supply to the fetus or its inability to use them. Therefore, it might be postulated that those neonates who have experienced intrauterine growth restriction can be expected to have an abnormal mineral content in their meconium.

This study aimed at substantiating the hypothesis that SGA neonates have lower meconium mineral concentration than appropriate for gestational age (AGA) neonates of similar GA. Toward this end, we specifically analyzed iron, zinc, copper, manganese, calcium, magnesium, and phosphorus.

PATIENT POPULATION AND METHODS

Meconium specimens were obtained from SGA and AGA neonates of similar GA born at North Shore University Hospital between July 2002 and April 2003, whose parent(s) agreed to participate without exclusion by maternal age, race, or prenatal medications. Consecutive SGA neonates born in that period were included irrespective of sex, head circumference, or multiple gestations. However, neonates with congenital anomalies of the gastrointestinal tract and chromosomal anomalies were excluded. Similarly, meconia were collected from AGA neonates matched as close as possible for GA and sex, as allowed by parental agreement. The race distribution for both groups closely matched that of the institution catchment area. SGA neonates were defined as those having a birth weight below the 10th percentile for GA. AGA neonates were defined as those having birth weight between the 10th and 19th percentile for GA.[5,13,14] Both SGA and AGA neonates were divided into two groups: those with ≤ 35 weeks GA and those ≥ 36 weeks GA to avoid extreme GA comparisons. Among the SGA infants ≤ 35 weeks GA, four were males and seven were females. Among the AGA neonates, there were seven males and eight females. Among those infants ≥ 36 weeks GA, there were six males and four females in the SGA group, and seven males and two females in the AGA neonates. Parental informed consent was obtained prior to enrolling the neonates in the study. The protocol was approved by the Institutional Review Board.

All excreta recognized as meconium by properly instructed nursing staff was removed with a wooden spatula from every diaper changed. Collection was discontinued when transitional stools were passed, characterized by their yellowish green color and nonviscous content. All samples from each neonate were placed in sterile plastic containers and frozen at −20°C, combined, and lyophilized overnight. The dried powder was weighed and triturated in a glass mortar. Duplicate aliquots (200 to 300 mg each) of the specimens were placed in 16 × 100 mm test tubes and digested with 2 mL of concentrated nitric acid (trace element grade, Fisher Scientific, Pittsburg, PA) at room temperature in a fume hood until the entire sample was dissolved. The acid solution was further diluted to 10 mL and filtered through analytical grade paper prior to analysis. Portions of the absorbent materials of unused diapers were extracted with acid as described and used as matrix blank to correct for exogenous mineral sources. Samples were assayed for iron, zinc, copper, manganese, calcium, and magnesium by atomic absorption spectrophotometry (SpectrAA10, Varian Instruments, Sunnyvale, CA) against certified external standards (Fisher Scientific). Phosphorus was determined by a colorimetric method.[15] Results were expressed as $\mu g \cdot g^{-1}$ dry meconium and also normalized in reference to birthweight and reported as $\mu g \cdot g^{-1} \cdot kg$ birthweight^{-1} to compensate for weight discrepancies.

Data were analyzed by one-way analysis of variance, if normally distributed, or by the Mann-Whitney test if the distributions were not normal,[16] using a computer program (SigmaStat, SPSS Inc., Chicago, IL). We accepted a threshold of significance of 0.05. Based on previous studies, we estimated that 10 neonates per group would be needed to detect a 25% difference in the mean of two groups for at least one of the elements, at 80% power, provided the pooled coefficient of variation was $\leq 20\%$.[17] Data are expressed as mean ± standstandard error of the mean.

Table 1 Meconium Mineral Concentration in ≤35 wk Gestational Age AGA and SGA Neonates

Element	AGA (n = 15) (μg·g^{-1})	SGA (n = 11) (μg·g^{-1})
Iron	186.4 ± 29.7	92.7 ± 15.2*
Zinc	482.8 ± 46.0	458.9 ± 65.5
Copper	115.8 ± 9.6	117.3 ± 24.4
Manganese	40.2 ± 5.8	24.1 ± 5.2†
Calcium	4397.9 ± 1585.3	2096.6 ± 455.8
Magnesium	3917.5 ± 318.5	3370.8 ± 429
Phosphorus	940 ± 396.4	514.2 ± 194.6

Data are expressed as mean ± standard error of the mean.
*$p < 0.02$.
†$p < 0.05$.
AGA, appropriate for gestational age; SGA, small for gestational age.

RESULTS

Meconium mineral concentrations are presented in Table 1 for neonates ≤35 weeks GA and in Table 2 for those ≥36 weeks GA. Iron and manganese concentrations were significantly lower in the SGA infants ≤35 weeks GA group. However, there were no differences for either of these two elements or for the remaining five others between SGA and AGA infants ≥36 weeks GA. Elements normally present at the highest concentrations in meconium, such as calcium, magnesium, and phosphorus, presented a wider dispersion of values. In addition, calcium/phosphorus molar ratios in meconium did not differ between AGA and SGA infants or by GA. For infants ≤35 weeks GA, the AGA ratio was 6.64 ± 1.32 (n = 15) and for the SGA infants, the AGA ratio was 5.79 ± 1.23 (n = 11). For the infants ≥36 weeks GA, the AGA ratio was 6.29 ± 1.49 (n = 9) and for the SGA infants, the AGA ratio was 4.26 ± 0.99 (n = 10). Further examination of the data revealed that among AGA neonates ≤35 weeks GA, there was a significant negative correlation between copper concentration and birthweight (Fig. 1; $r = -0.715$, $p = 0.003$; 13 degrees of freedom). The other elements did not present significant relationships.

Normalization of data taking into consideration birthweight still showed a difference in meconium iron, but not in meconium manganese, in the newborns ≤35 weeks GA (Table 3). In contrast, birthweight-adjusted meconium copper was higher in the ≥36 weeks GA SGA infants than in the AGA infants of similar GA (Table 4).

Table 2 Meconium Mineral Concentration in ≥36 wk Gestational Age AGA and SGA Neonates

Element	AGA (n = 9) (μg·g^{-1})	SGA (n = 10) (μg·g^{-1})
Iron	89.4 ± 13.0	79.1 ± 12.6
Zinc	667.7 ± 158.0	456.1 ± 95.3
Copper	79.7 ± 16.0	93.6 ± 11.0
Manganese	25.4 ± 4.4	24.7 ± 3.5
Calcium	1410.1 ± 268.2	1156.6 ± 356.4
Magnesium	3196.8 ± 506.7	3179.9 ± 492.3
Phosphorus	216.4 ± 52.2	271.4 ± 97.2

Data are expressed as mean ± standard error of the mean.
AGA, appropriate for gestational age; SGA, small for gestational age.

DISCUSSION

These data show that the ≤35 week SGA infants had less meconium iron and manganese than AGA neonates of comparable GA. This may be due to a decrease in nutrient supply or increased use of these elements by the fetus.[12] The absence of neonatal anemia does not suggest that iron stores are adequate, given that red blood cells are produced in preference to storing iron in all other organs of the body, especially the liver. Similarly, hepatic iron stores are lost in preference to cardiac and brain stores, as shown in postmortem examinations of infants who died as a result of utero-placental insufficiency.[18] In the rat, the developing hippocampus has been shown to be vulnerable to iron deficiency.[19] This may have implications for the development of the human brain as well. Preterm newborns have proportionally lower iron stores than term neonates.[20]

The pattern of manganese concentration changes in SGA and AGA neonates was similar to those of iron; that is, the SGA infants showed significantly lower manganese concentrations at ≤35 weeks GA, but no differences between neonates born ≥36 weeks GA. However, when birthweights were taken into account, the differences between the two groups became indistinguishable. Manganese is a constituent of key enzymes in free radical scavenging and carbohydrate metabolism, respectively.[21] Reduced concentration of manganese in meconium may suggest inadequate stores of this element in the preterm SGA infant and possible metabolic handicaps.

The molar calcium/phosphorus ratio obtained in meconium of both AGA and SGA infants of all GA far exceeds total body composition ratios reported for newborn infants.[22] This ratio also exceeds the calcium/phosphorus ratio in maternal plasma, a finding consistent with a greater intrauterine assimilation of phosphorus than of calcium.[8,13] A small amount of calcium, in addition to a preponderance of sodium and potassium, is present in amniotic fluid.[23] There is evidence of early development of small intestinal absorptive functions, which attests to the physiologic precocity of the human infant, including the premature infant.[23] However, amniotic fluid is generated by fetal urine and lung liquid, and is rapidly recycled though fetal swallowing and intramembranous absorption.[24]

The greater birthweight-adjusted meconium copper concentration in the SGA newborns ≥36 weeks GA is in line with the inverse relationship between birthweight and meconium copper concentration observed in the AGA group ≤35 weeks GA. This may reflect a

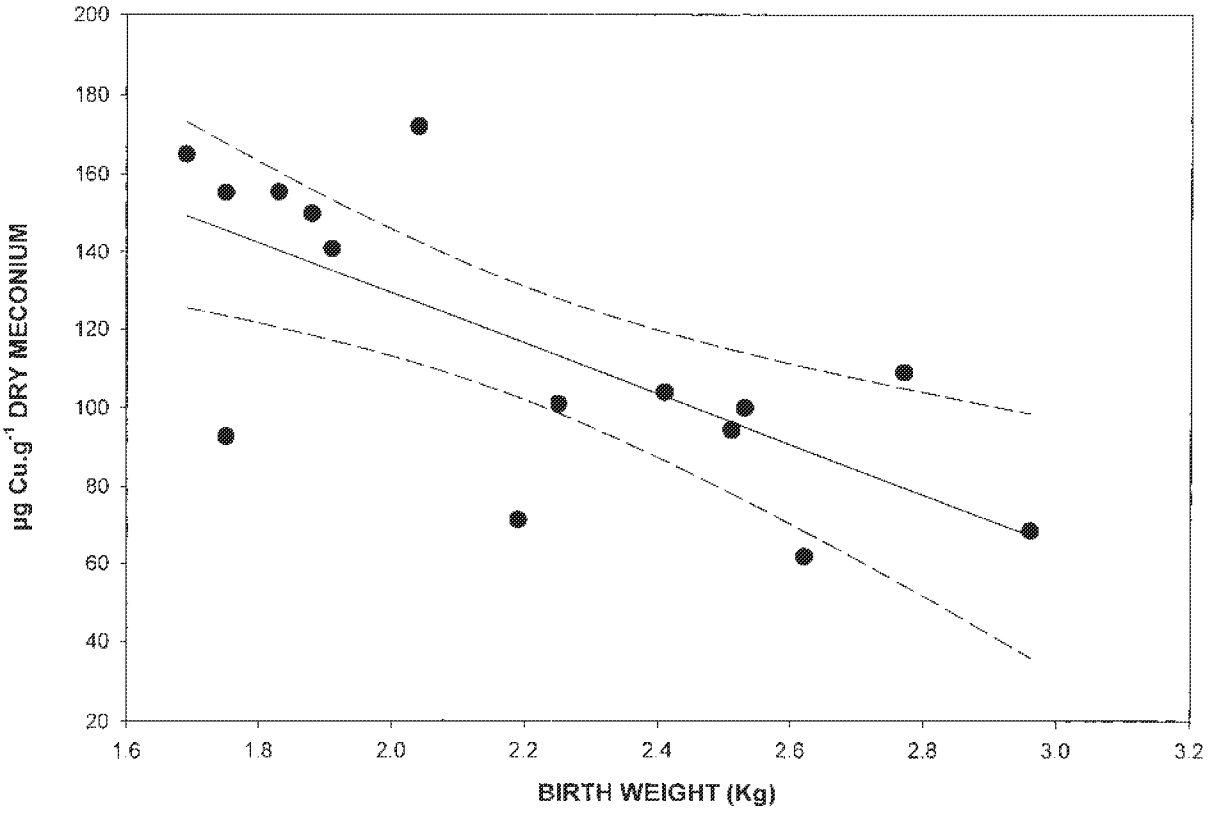

Figure 1 Correlation between meconium copper concentration and birthweight in appropriate for gestational age newborn infants born at ≤35 weeks gestational age. The regression line corresponds to $r = -0.715$, $p = 0.003$. The bands represent the 95% confidence intervals.

poorer use of this element by the SGA than by the AGA infant, or a relative oversupply provided by the mother, consistent with the known downhill gradient of copper across the placenta.[25,26]

The data in this study for AGA infants follows the same pattern as the results presented in a preceding report,[9] in which only AGA newborns were included. For these infants, iron, copper, calcium, and phosphorus concentrations were higher in the ≤35 weeks GA infants than in the ≥36 weeks GA infants (one-tail, $p < 0.01$, 0.05, 0.05 and 0.05, respectively). This decreasing mineral concentration trend with increasing GA could imply a greater use of those elements later in gestation, or a dilution effect due to accumulation of organic matter in meconium. In contrast, this concentration decrease trend was not present among SGA newborns, whether they were born at ≤35 or ≥36 weeks of GA, which substantiates

Table 3 Birthweight-Adjusted Meconium Mineral Concentration in ≤35 wk Gestational Age AGA and SGA Neonates

Element	AGA (n = 15) (µg·g^{-1}·kg birthweight^{-1})	SGA (n = 11) (µg·g^{-1}·kg birthweight^{-1})
Iron	117.3 ± 29.1	50.8 ± 8.7*
Zinc	235.7 ± 31.3	252.7 ± 36.3
Copper	56.3 ± 6.6	64.0 ± 13.0
Manganese	19.5 ± 3.2	13.3 ± 2.9
Calcium	2037.7 ± 741.6	1133.8 ± 224.6
Magnesium	1878.3 ± 208.5	1849.2 ± 238.8
Phosphorus	448.9 ± 197.8	277.4 ± 99.1

Data are expressed as mean ± standard error of the mean.
*$p < 0.05$.
AGA, appropriate for gestational age; SGA, small for gestational age.

Table 4 Birthweight-Adjusted Meconium Mineral Concentration in ≥36 wk Gestational Age AGA and SGA Neonates

Element	AGA (n = 9) (µg·g^{-1}·kg birthweight^{-1})	SGA (n = 10) (µg·g^{-1}·kg birthweight^{-1})
Iron	29.7 ± 5.4	35.1 ± 5.7
Zinc	217.7 ± 54.5	210.5 ± 48.1
Copper	26.7 ± 6.7	42.5 ± 6.3*
Manganese	8.2 ± 1.6	11.3 ± 1.8
Calcium	456.8 ± 97.9	507.5 ± 153.3
Magnesium	1012.4 ± 165.7	1452.3 ± 235.0
Phosphorus	66.3 ± 13.3	124.7 ± 48.0

Data are expressed as mean ± standard error of the mean.
*$p < 0.05$.
AGA, appropriate for gestational age; SGA, small for gestational age.

differences in mineral use between SGA and AGA fetuses in utero.

Analysis of meconium offers potential for examining mineral metabolism during gestation because it provides a record of accumulated terminal products present in the gastrointestinal tract of the newborn. This possibility has only been incompletely explored, although it has the merit of being noninvasive and readily accessible. Meconium analysis has been proposed as a way to detect intrauterine illicit drug exposure.[27] Meconium may be useful in assessing the nutritional needs of the newborn in the perinatal period and potentially assist investigators in designing optimal nutritional strategies for the preterm infant.

As the GA of viable infants is shortened, meconium analysis of these newborns may reveal information on the placental transport of minerals that now can only be theorized. Such information and a better understanding of the mineral status of the SGA infants, especially if born prematurely, may provide a guide for increased administration of selective mineral elements to pregnant women at risk, as well as to SGA newborns, who may experience subclinical nutritional deficiencies. In addition, meconium mineral analysis may also contribute to the evaluation of nutritionally deprived newborns in certain populations.

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